

Title: TARGET-DEPENDENT TRANSCRIPTION USING  
 DELETION MUTANTS OF N4 RNA POLYMERASE  
 Inventor(s): Elena K. Davydova/Lucia B. Rothman-Denes/Gary A. Dahl/  
 Svetlana Y. Gerdes/Jerome J. Jendrisak  
 Application No.:  
 Docket Number: 310307.00006

1/29

# Bacteriophage N4 vRNAP promoters

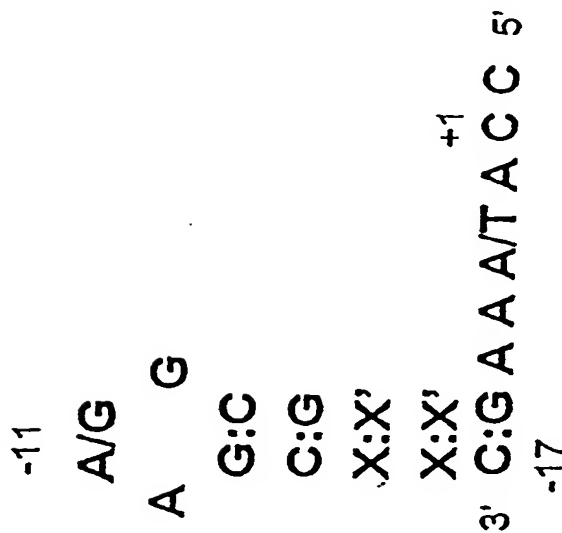


FIG. 1

## N4 vRNAP and generation of mini-vRNAP

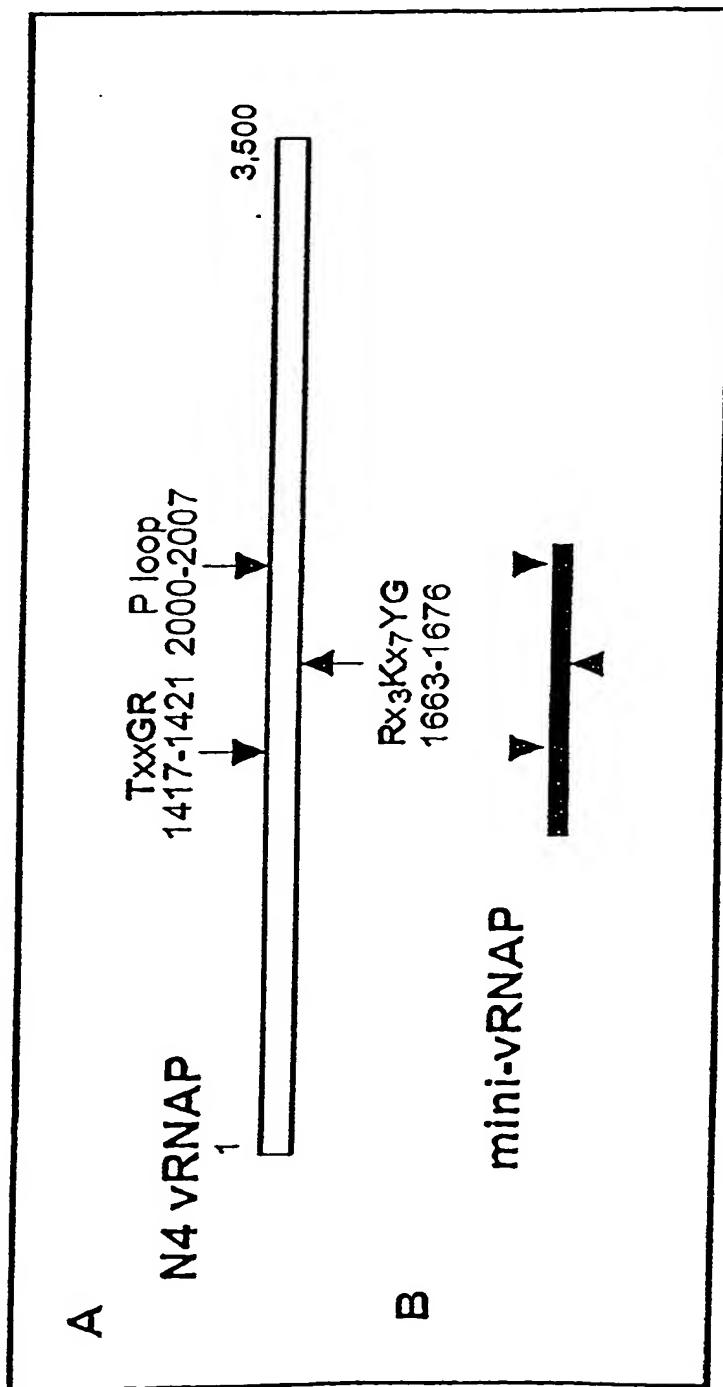


FIG. 2

Identification of the minimal active domain of N4 vRNAP  
 by proteolytic cleavage.

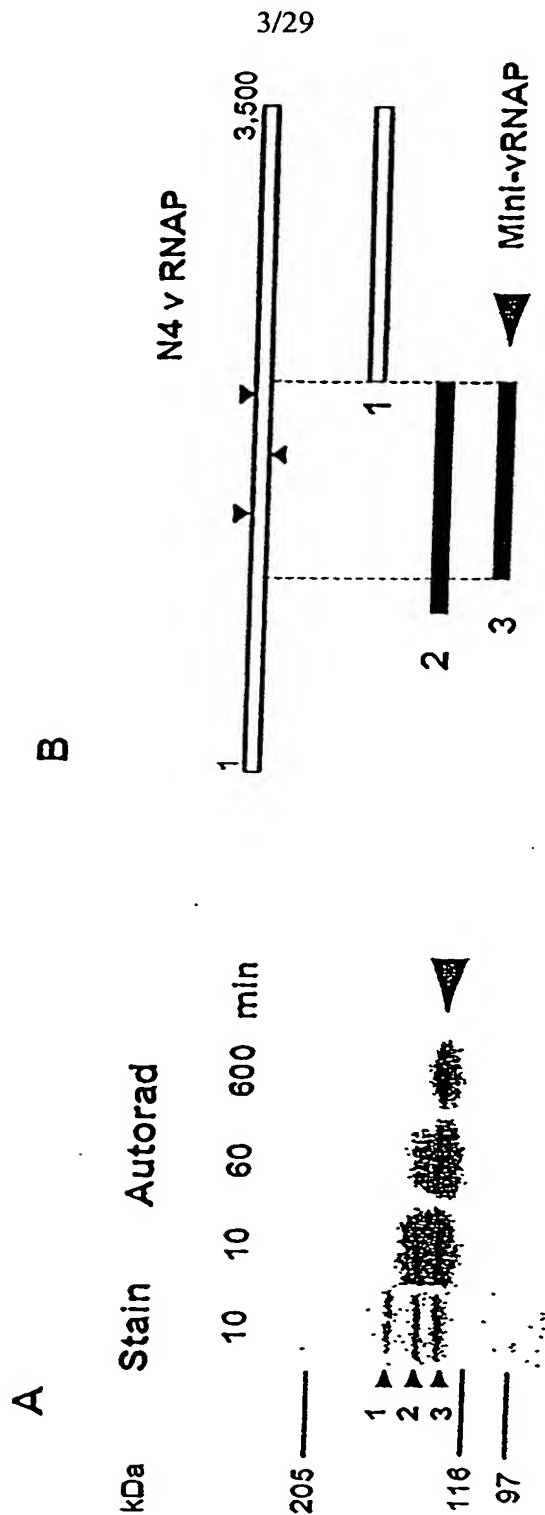
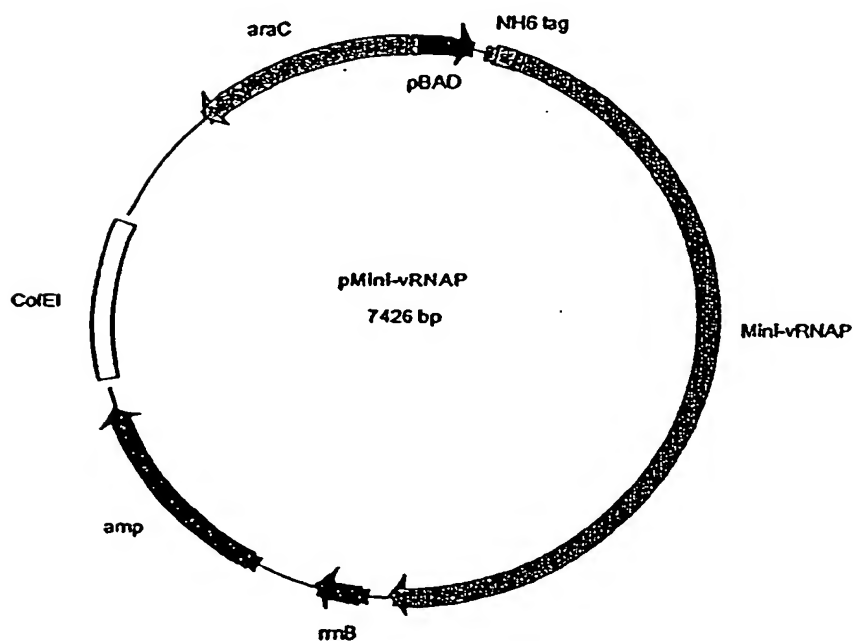


FIG. 3

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4/29



Plasmid name: pMini-vRNAP  
Plasmid size: 7426 bp  
Constructed by: K. M. Kazmierczak  
Construction date: 2/2000  
Comments: Insert cloned into Invitrogen  
pBAD B expression plasmid

FIG 4

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Application No.:  
Docket Number: 310307.00006

5/29

### Purification of cloned vRNAP and mini-vRNAP

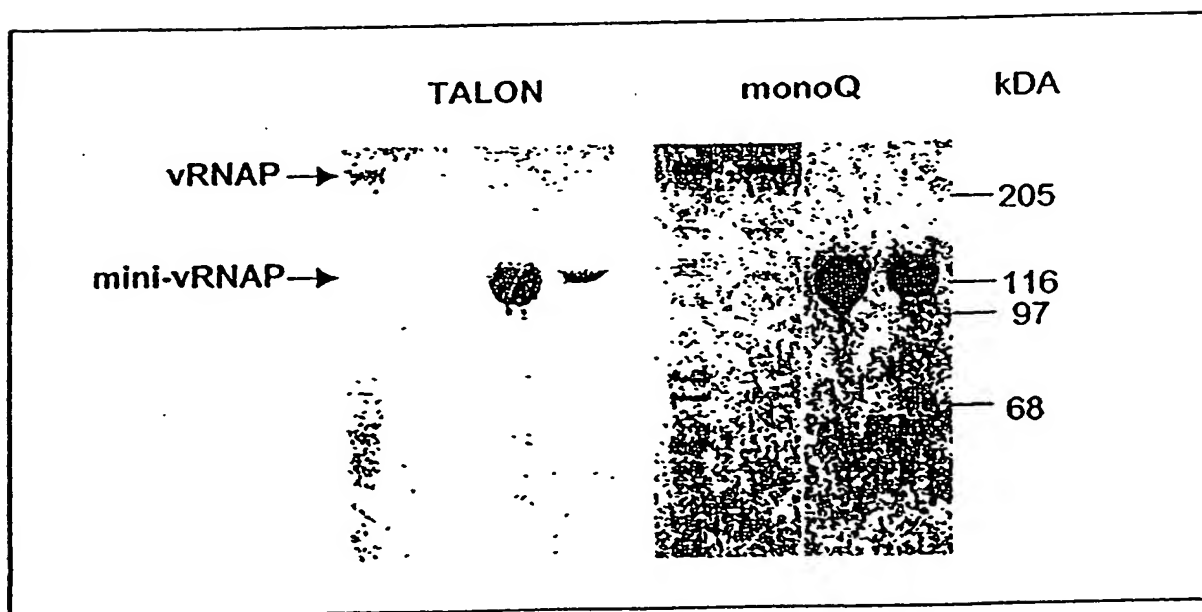


FIG 5

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Docket Number: 310307.00006

6/29

### Activation of N4 vRNAP transcription by *Eco* SSB at different ssDNA concentrations

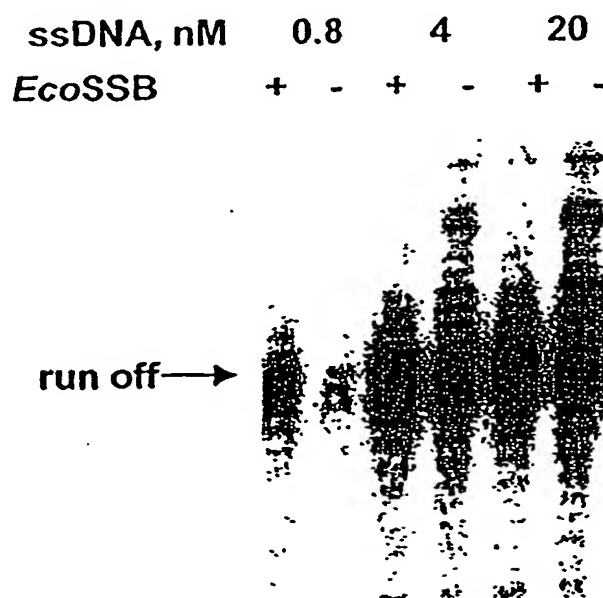


FIG 6

7/29

Effect of *Eco* SSB on ssDNA template recycling

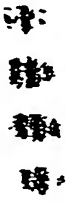
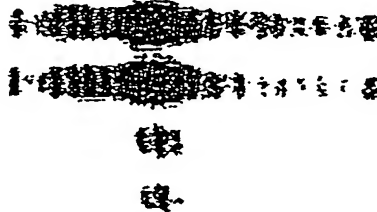
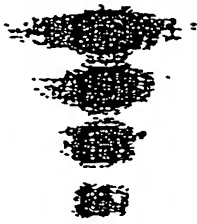
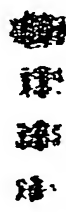
	A	B	C	D
Extra ssDNA	-	+	-	+
<i>Eco</i> SSB	-	-	+	-
time at 37°C	10' 20' 30' 40'	10' 20' 30' 40'	10' 20' 30' 40'	10' 20' 30' 40'
				

FIG. 7

8/29

Effect of *Eco* SSB on the state of template DNA  
 and product RNA in vRNAP transcription

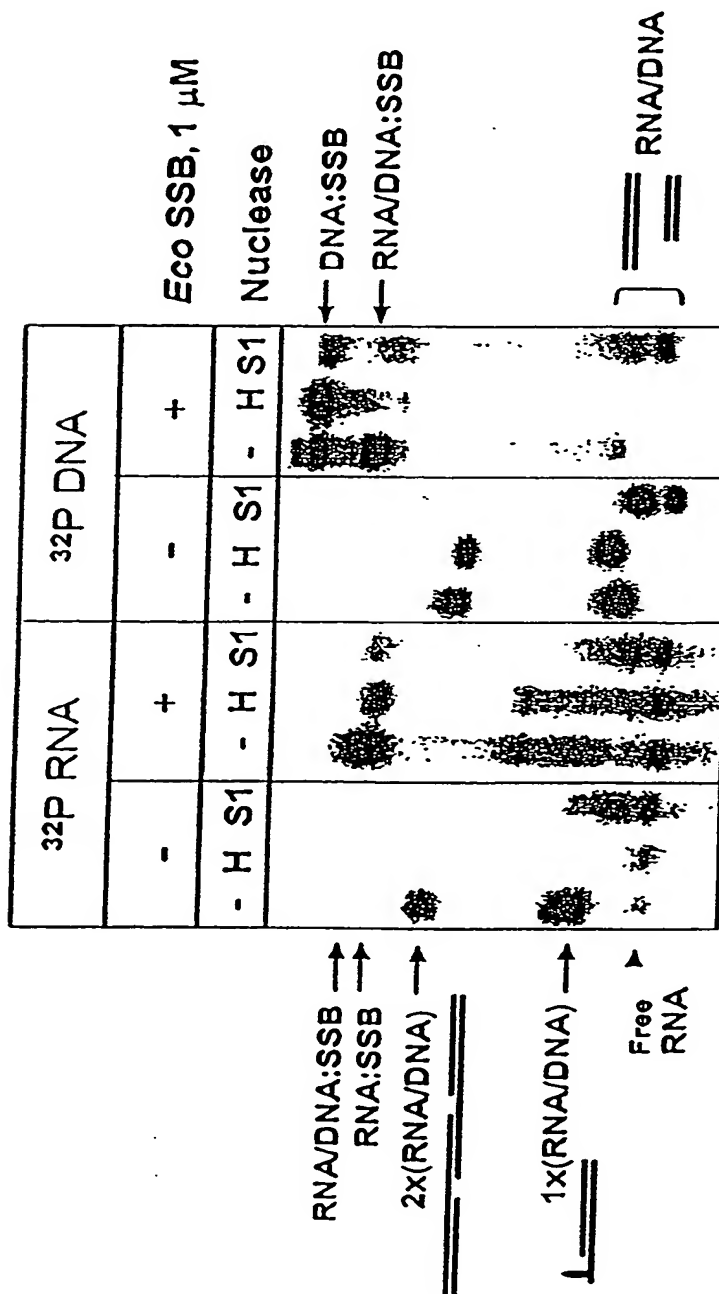


FIG. 8



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Application No.:

Docket Number: 310307.00006

9/29

Transcription initiation by vRNAP and mini-vRNAP

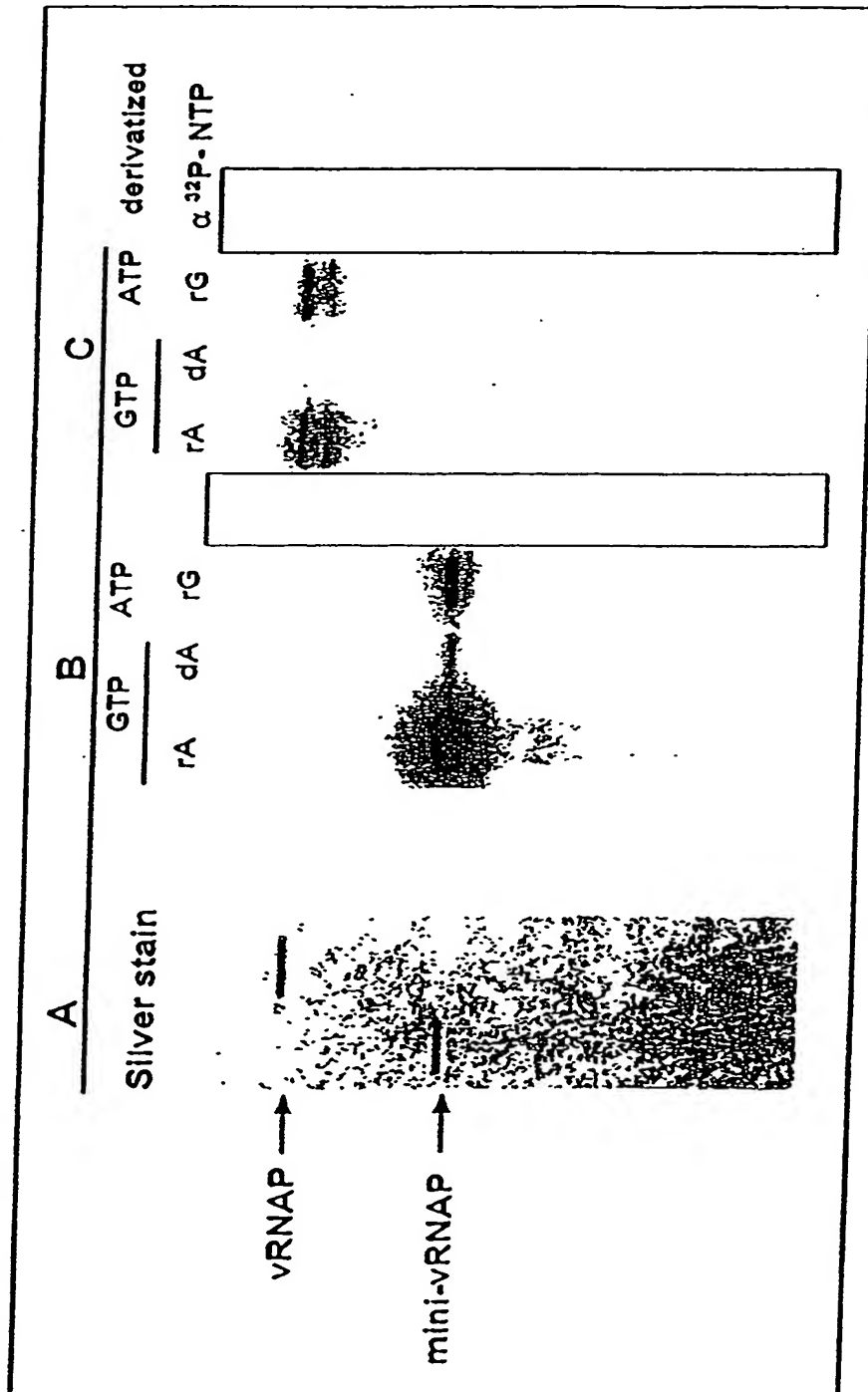


FIG. 9

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Application No.:

Docket Number: 310307.00006

10/29

# Effect of Eco SSB on transcription of vRNAP and mini-RNAP

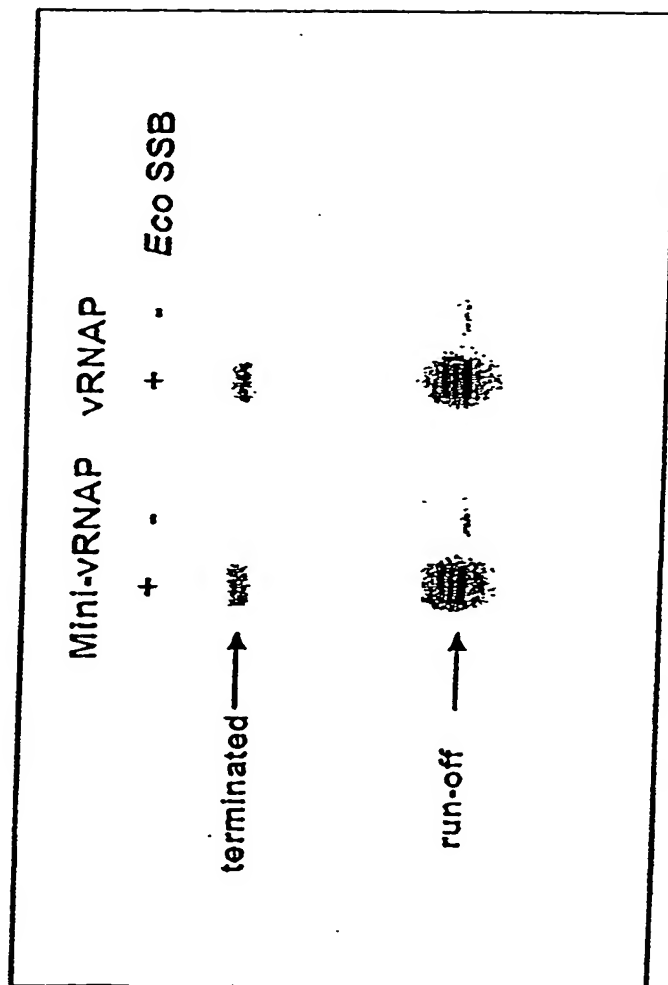


FIG. 10



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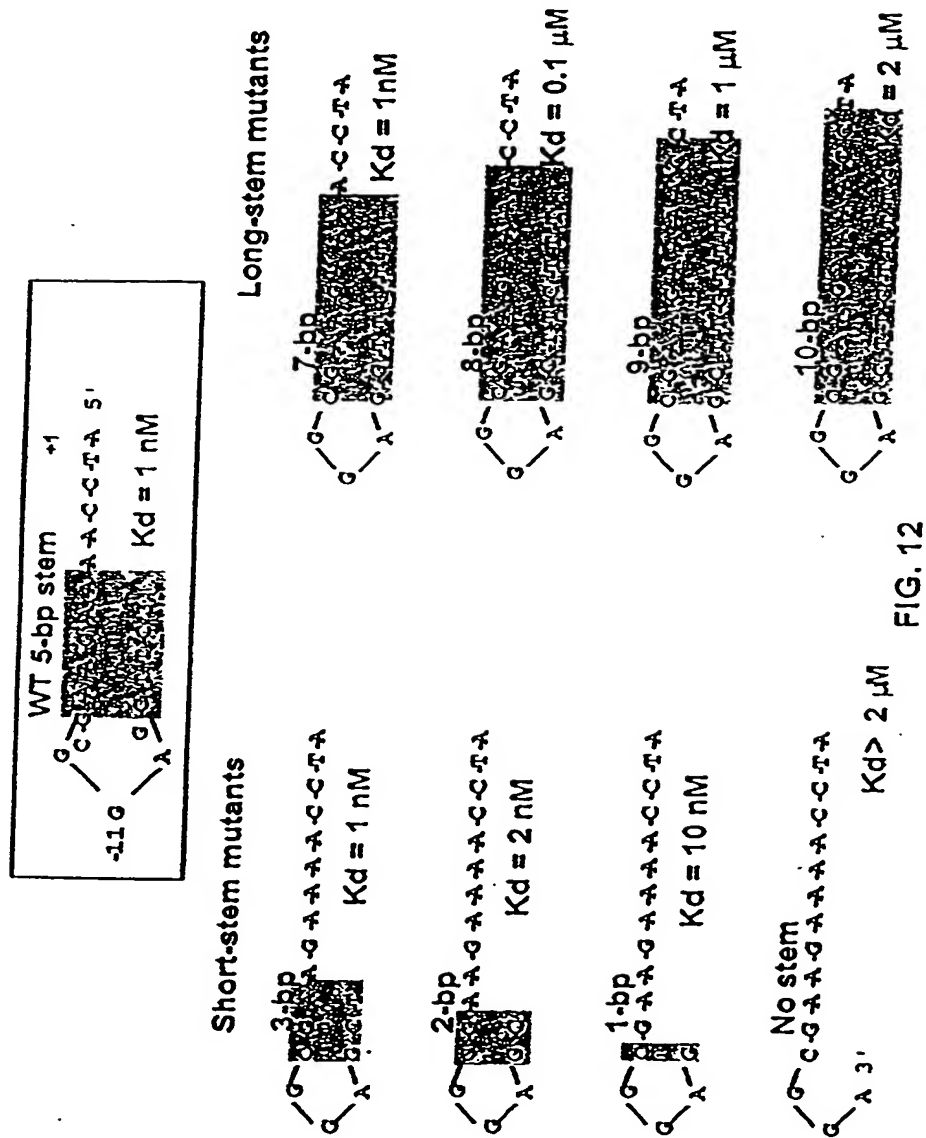
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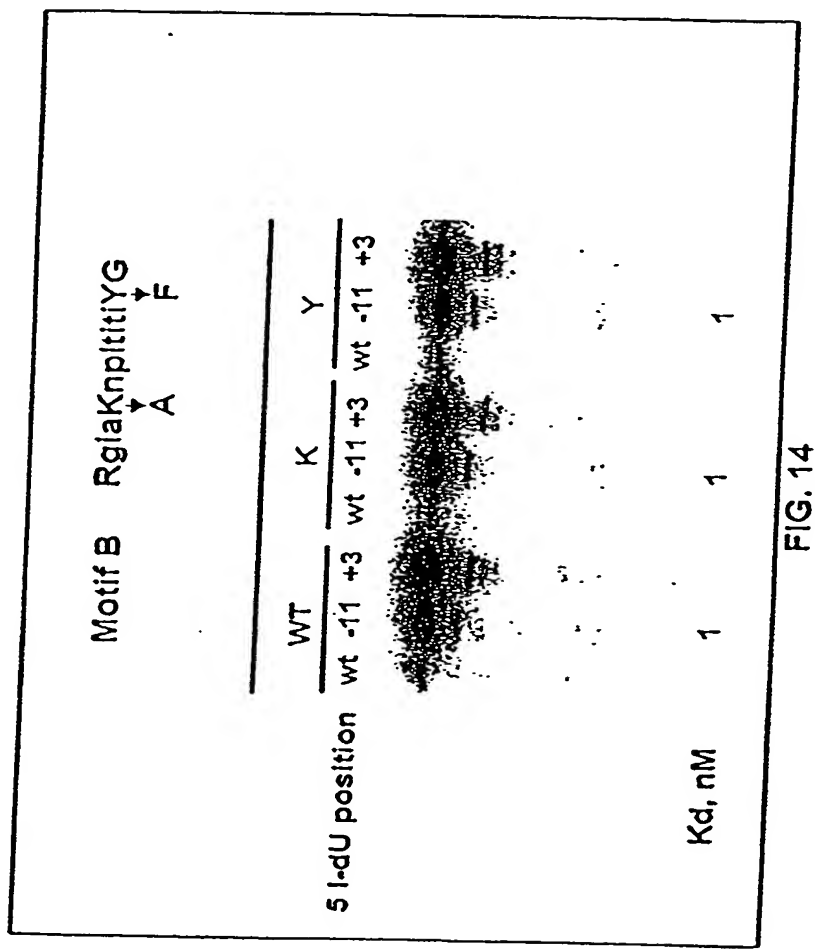
12/29

Binding affinities of stem-length promoter mutants



**FIG. 13**

UV crosslinking of mutant mini-vRNAPases  
 to promoter oligonucleotides



Run-off transcription by mutant mini-vRNAPases

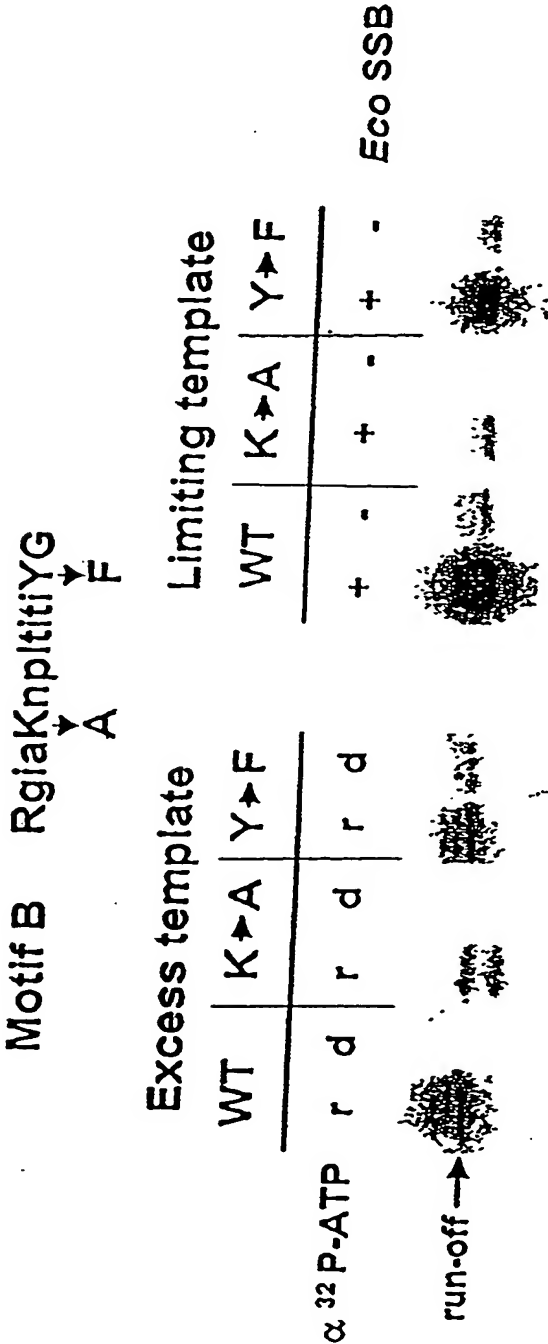


FIG. 15

16/29

# Mutant mini-vRNAPases transcription initiation

Motif B RgiaKnpltititY  
 A F



FIG. 16



17/29

Detection of *in vivo* activities of N4 vRNAP and mini-vRNAP

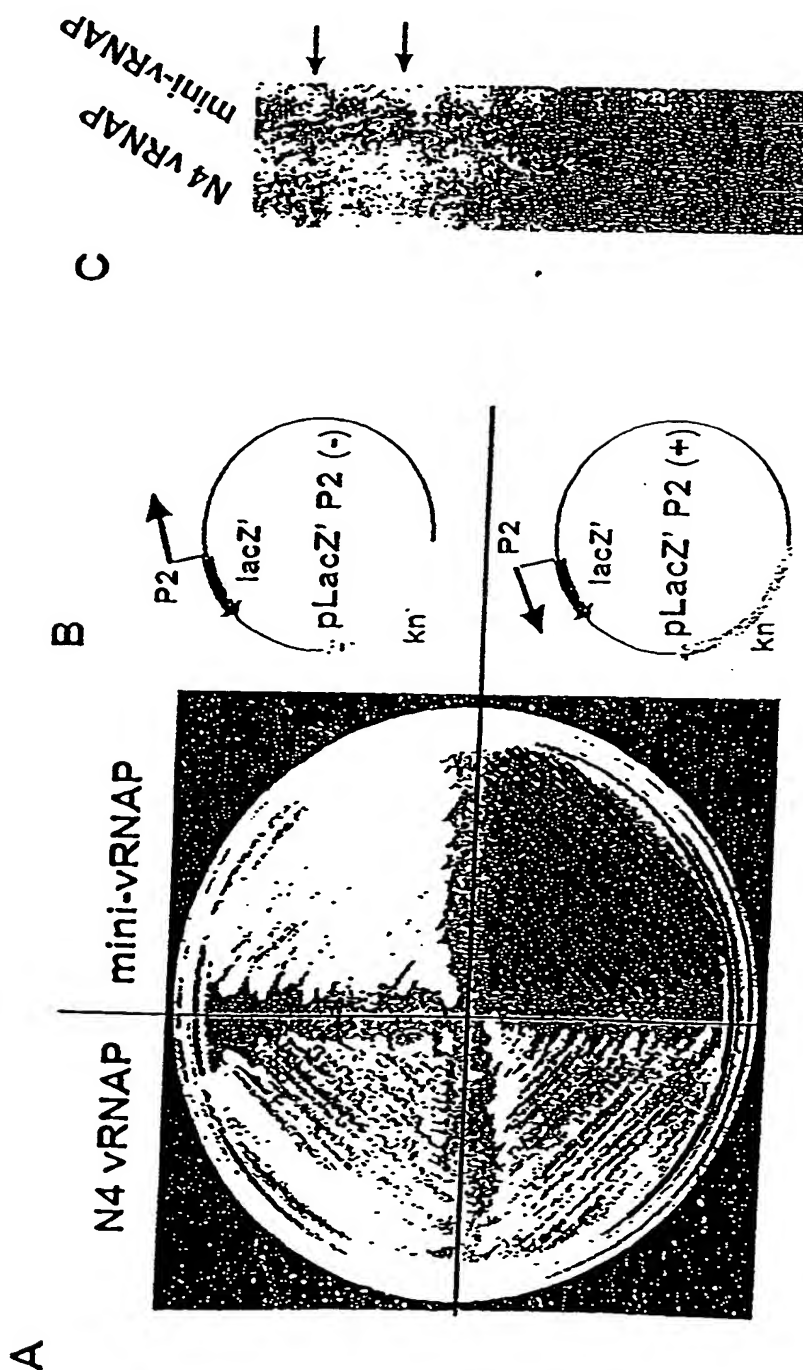
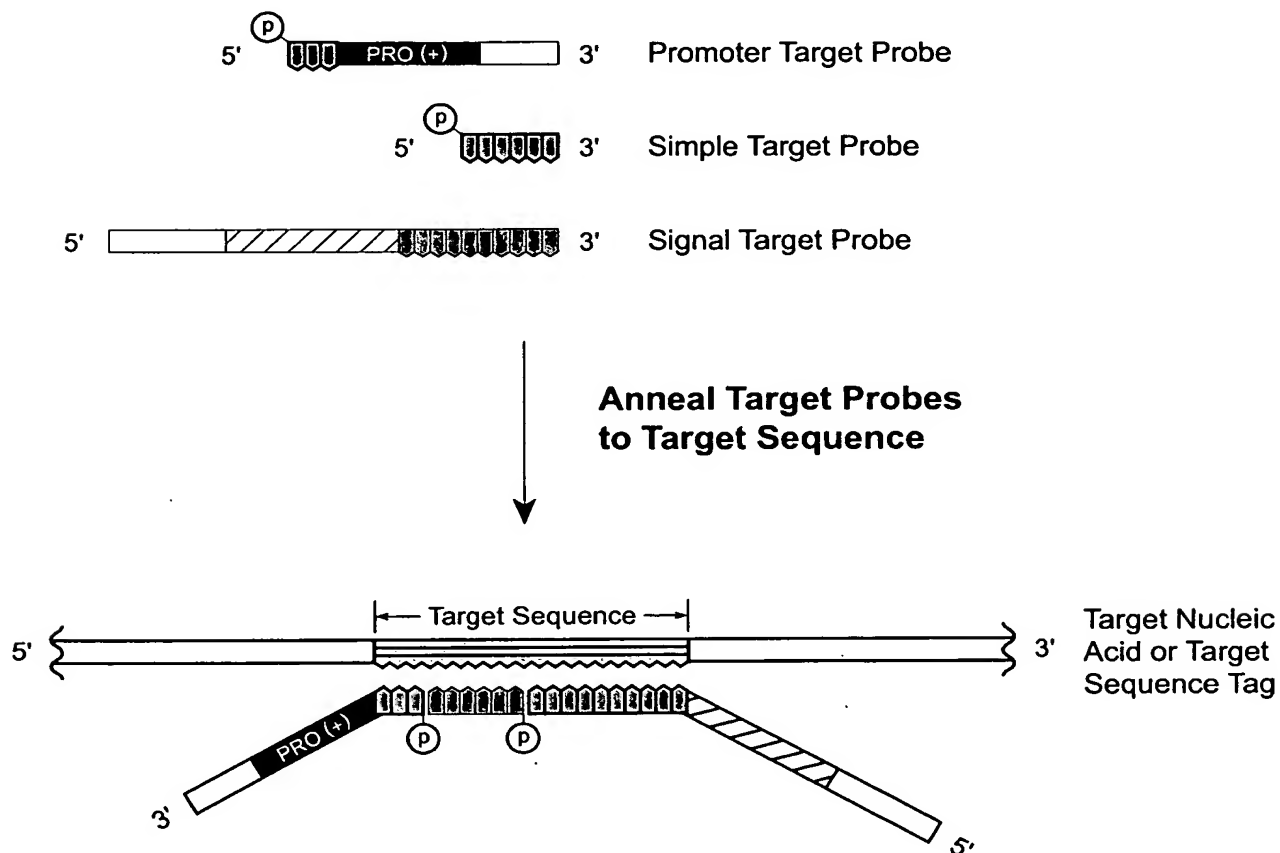


FIG. 17

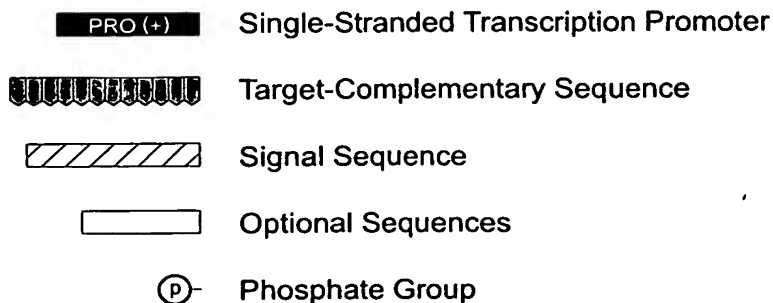
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 Docket Number: 310307.00006

18/29

### Monopartite Target Probes



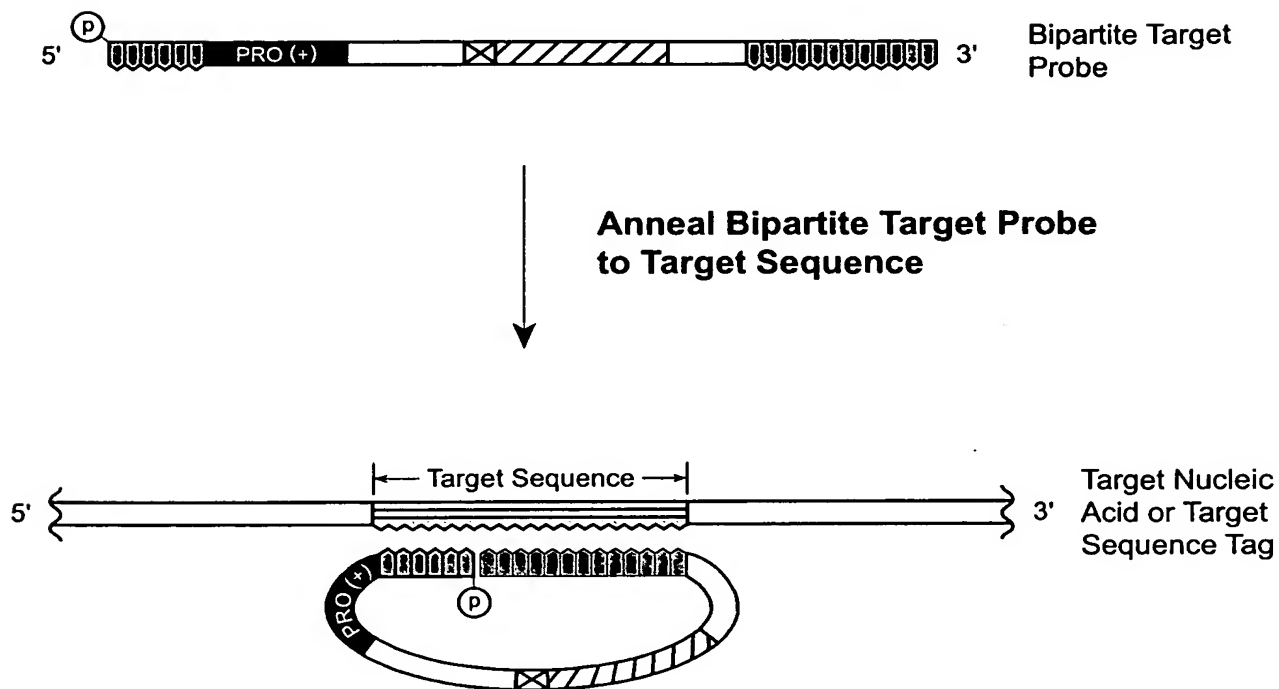
### Portions of a Monopartite Target Probe



**FIG 18**

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 Application No.:  
 Docket Number: 310307.00006

19/29



### Portions of a Bipartite Target Probe

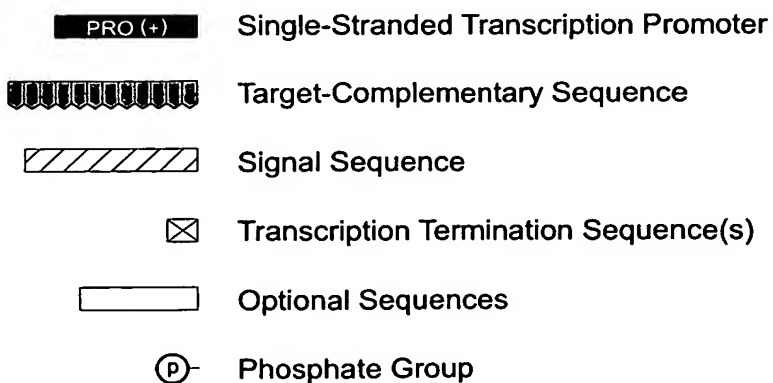


FIG 19

20/29

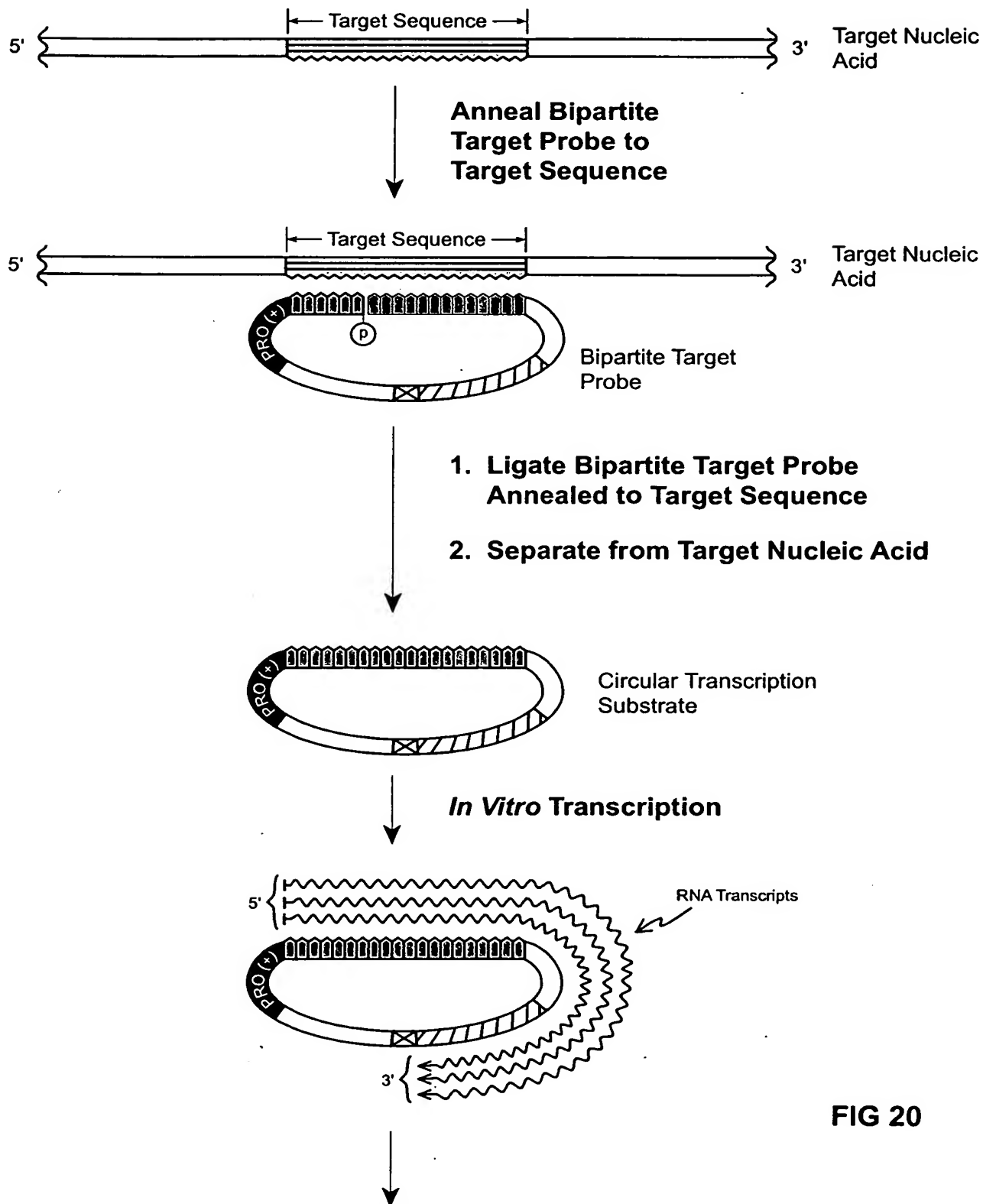
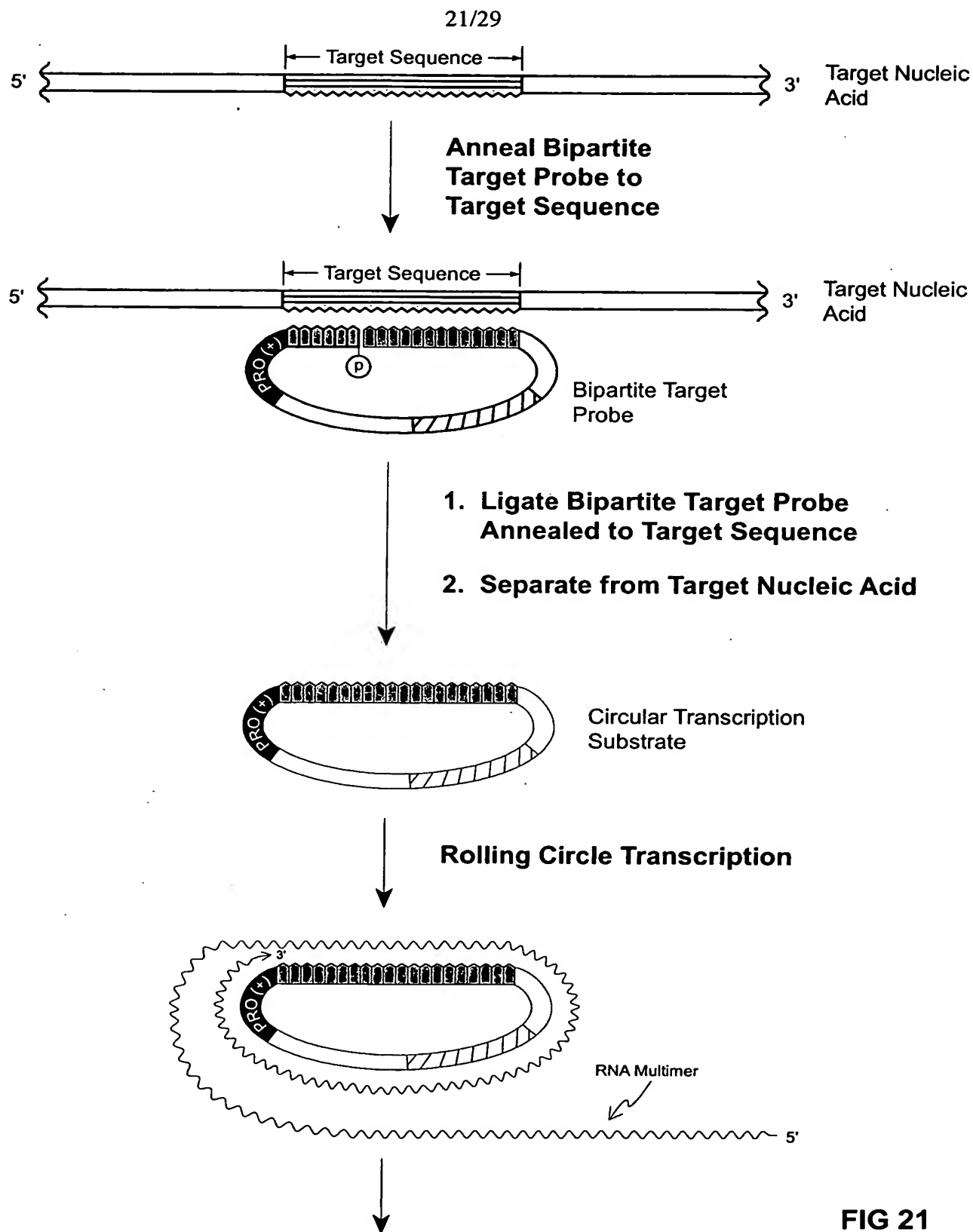


FIG 20

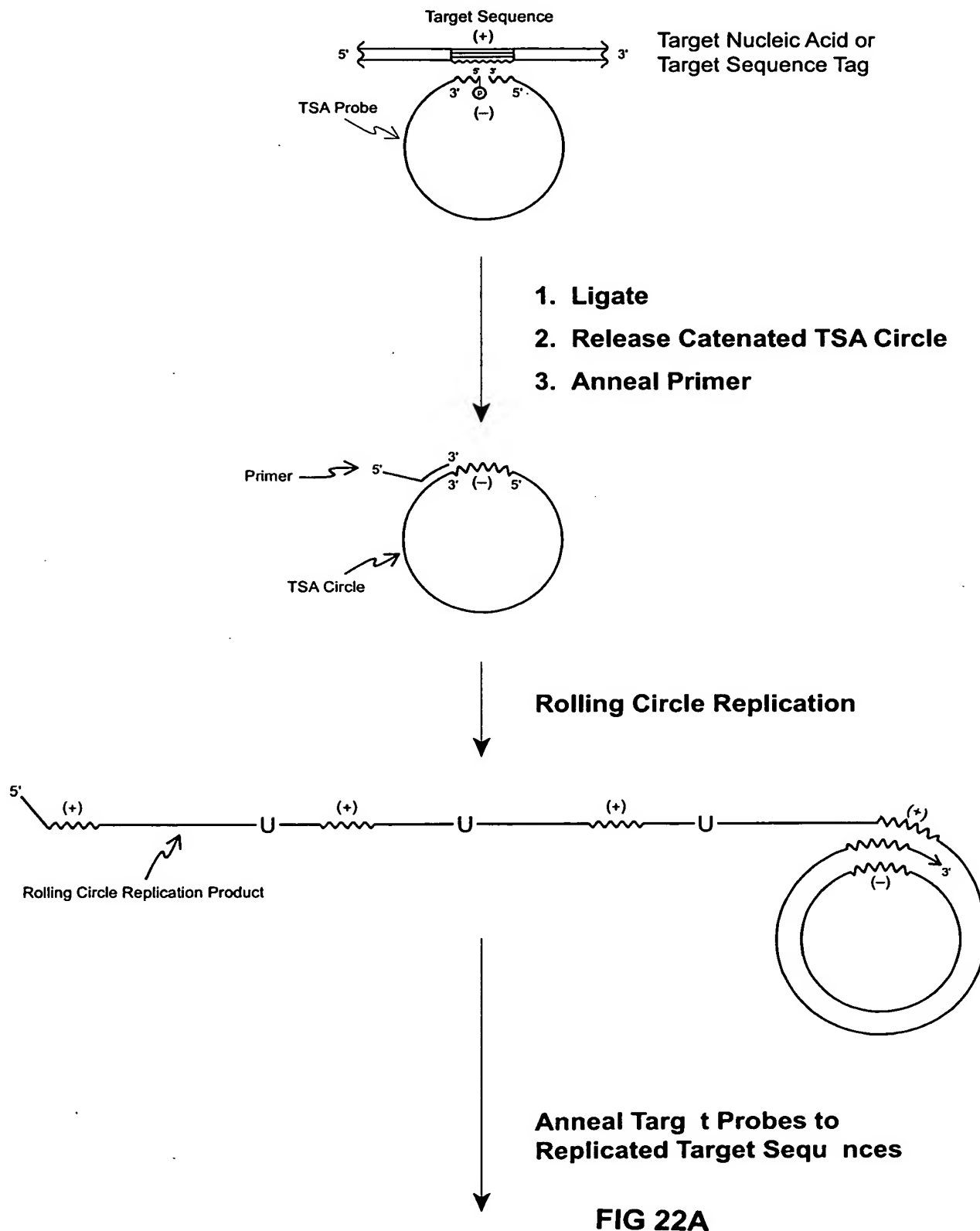


**FIG 21**

**Detect RNA Transcription Products Directly or Indirectly**

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22/29



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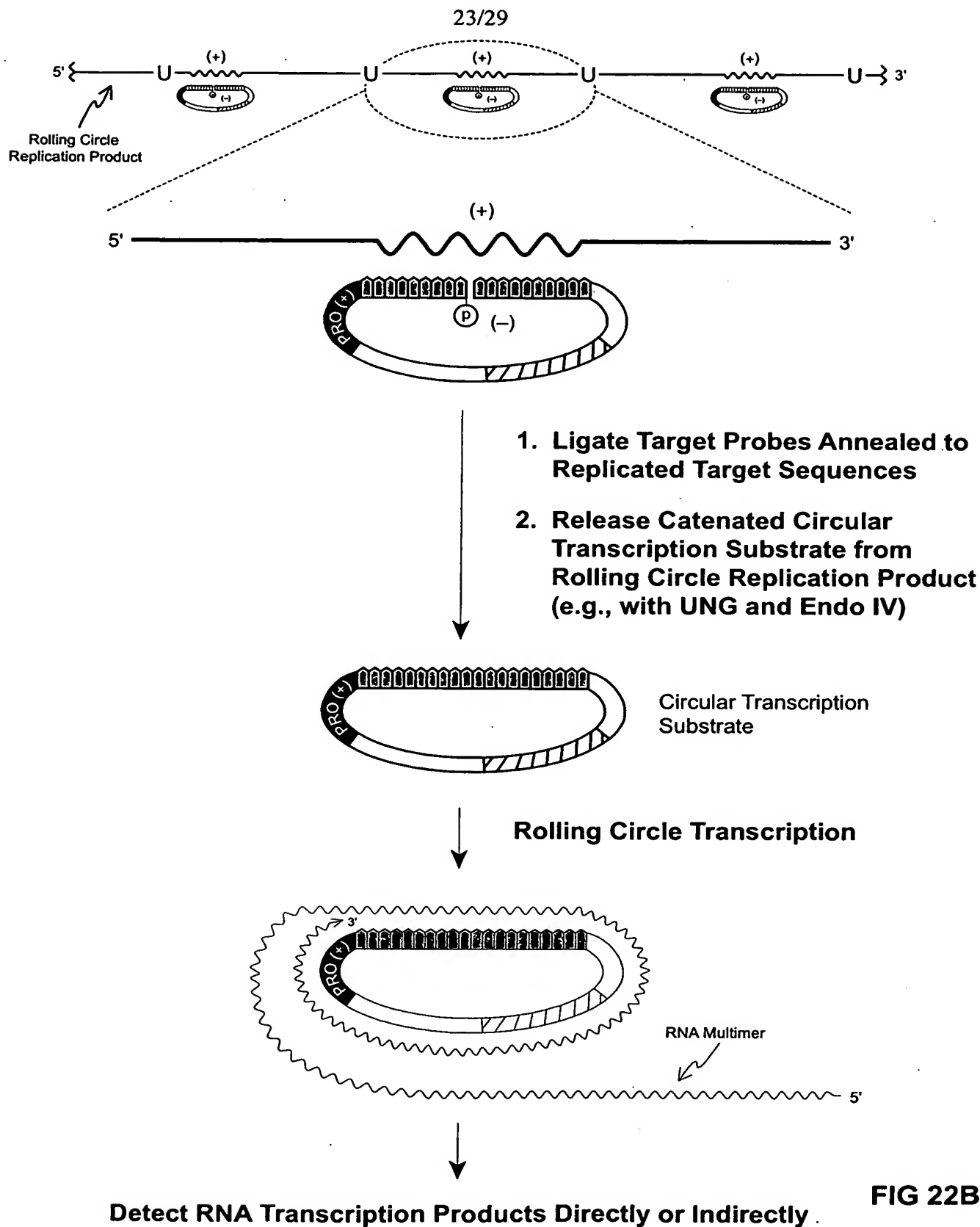


FIG 22B

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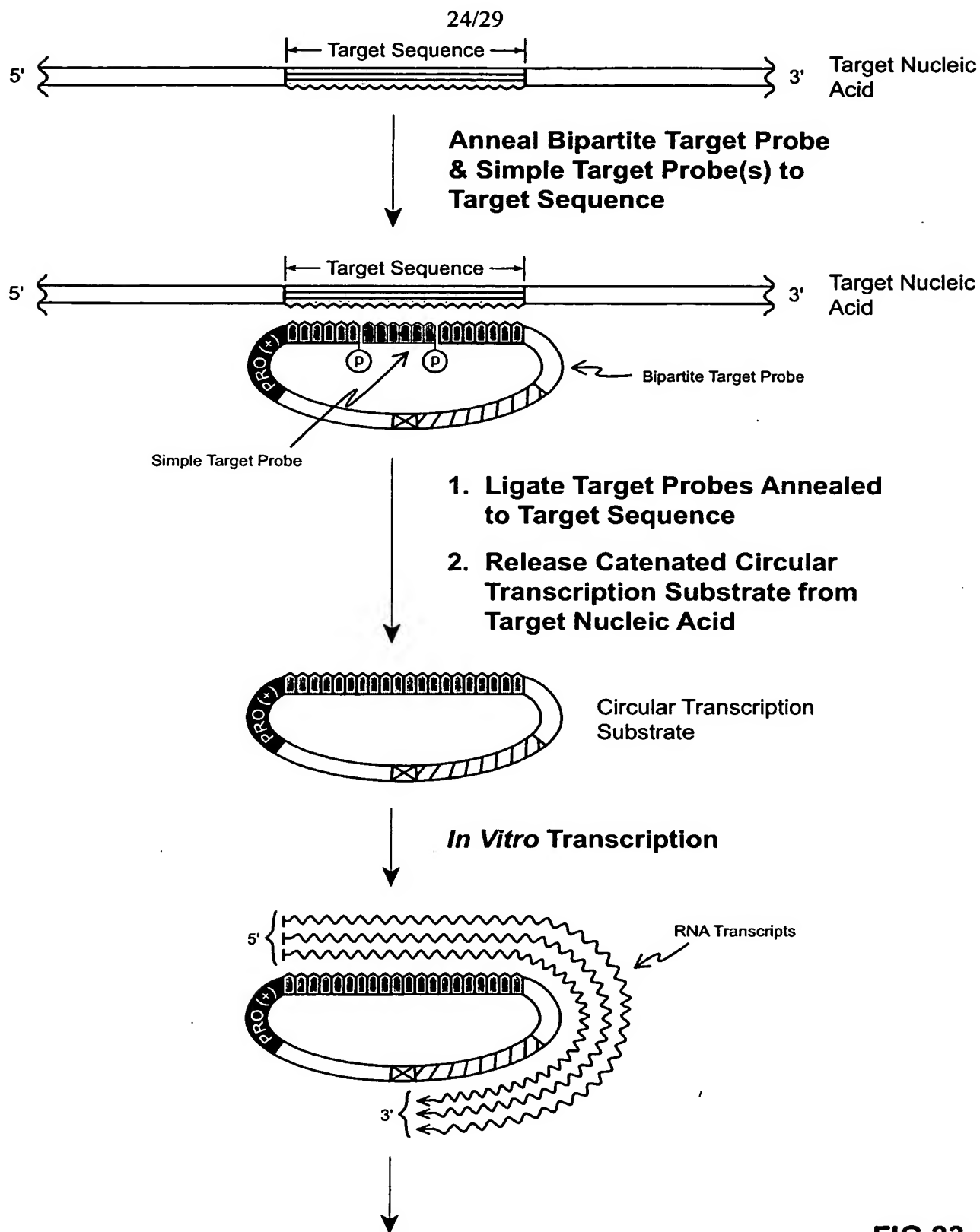
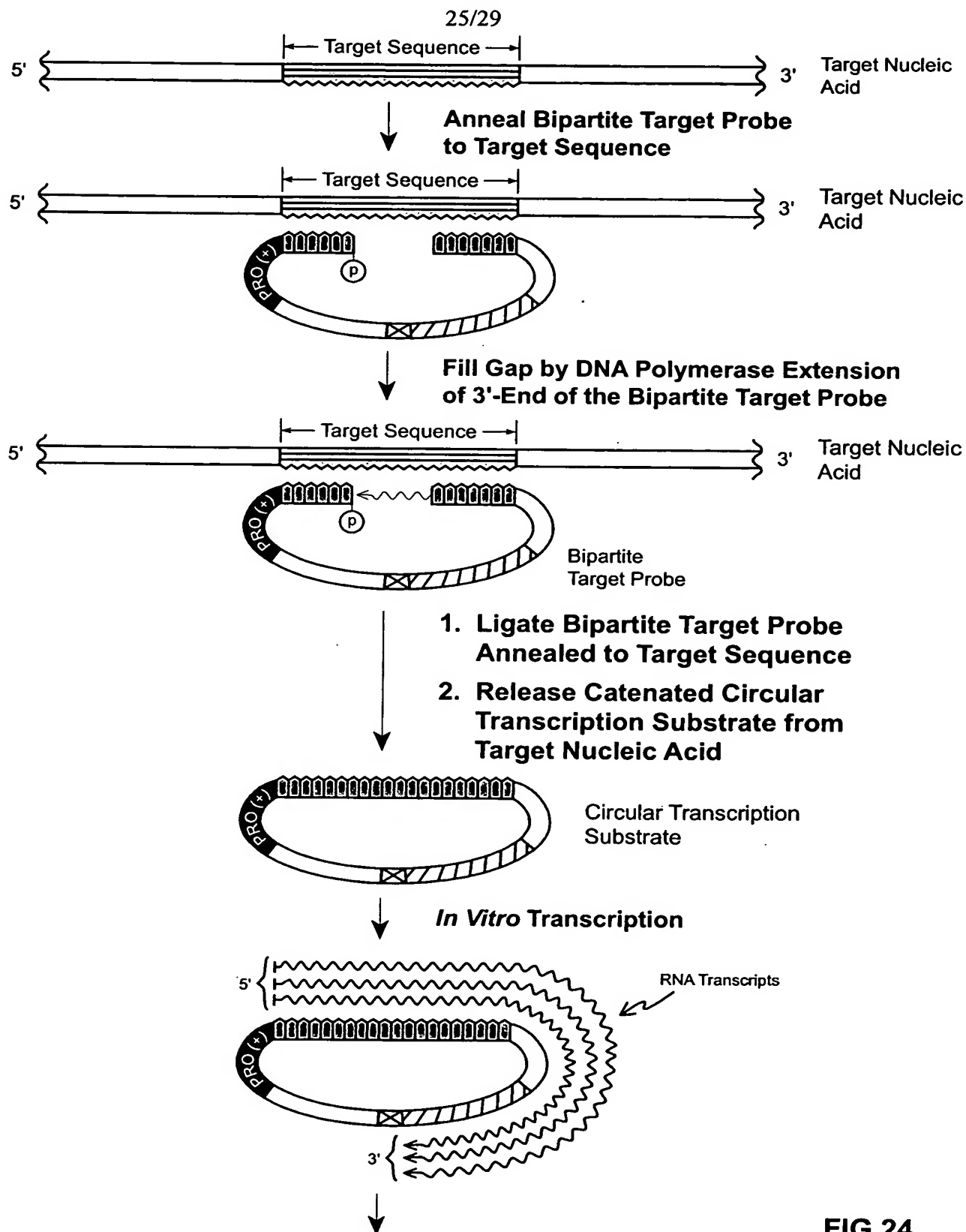


FIG 23

Detect RNA Transcription Products Directly or Indirectly



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**FIG 24**

**Detect RNA Transcription Products Directly or Indirectly**

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Application No.:  
Docket Number: 310307.00006

26/29

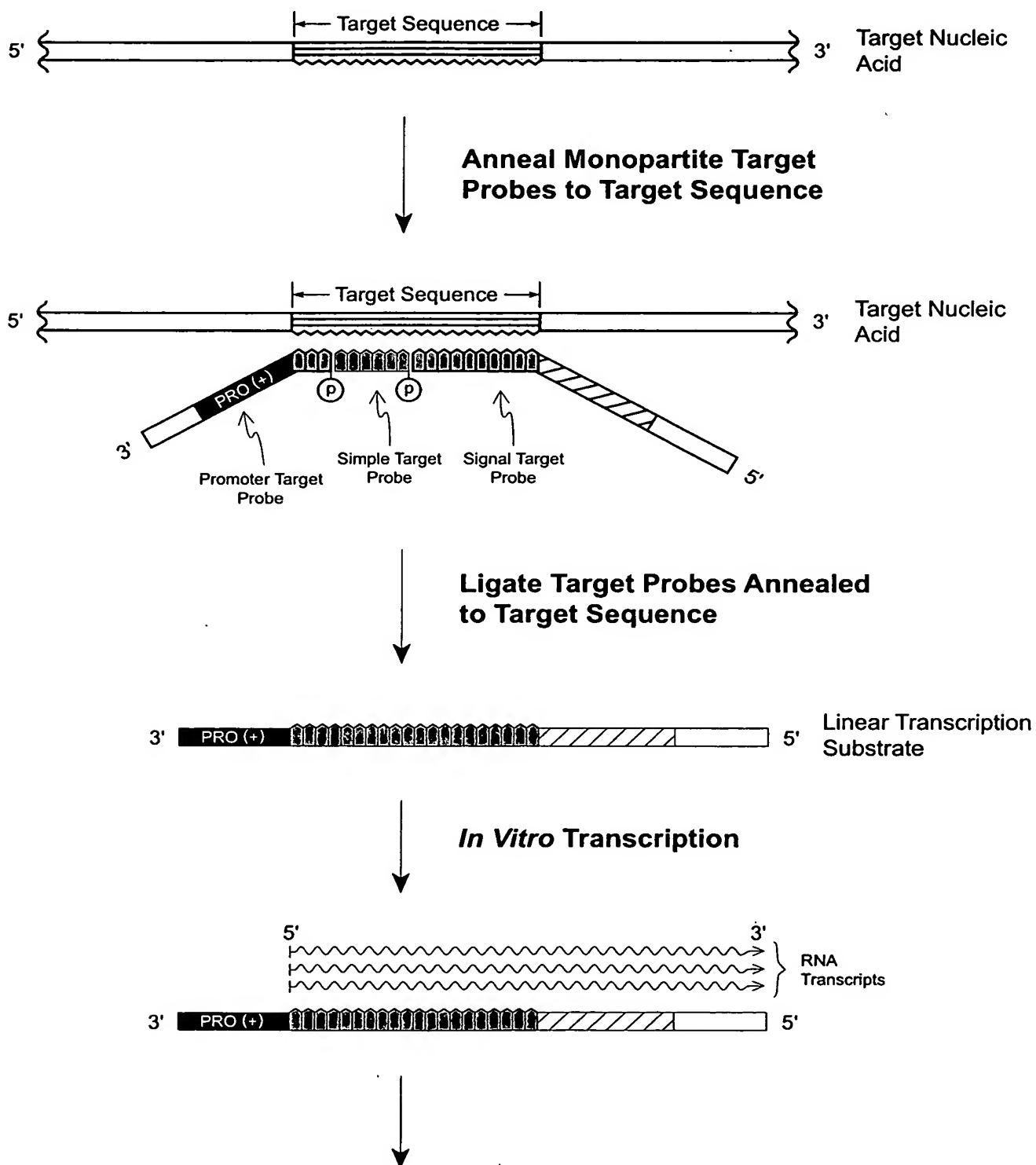
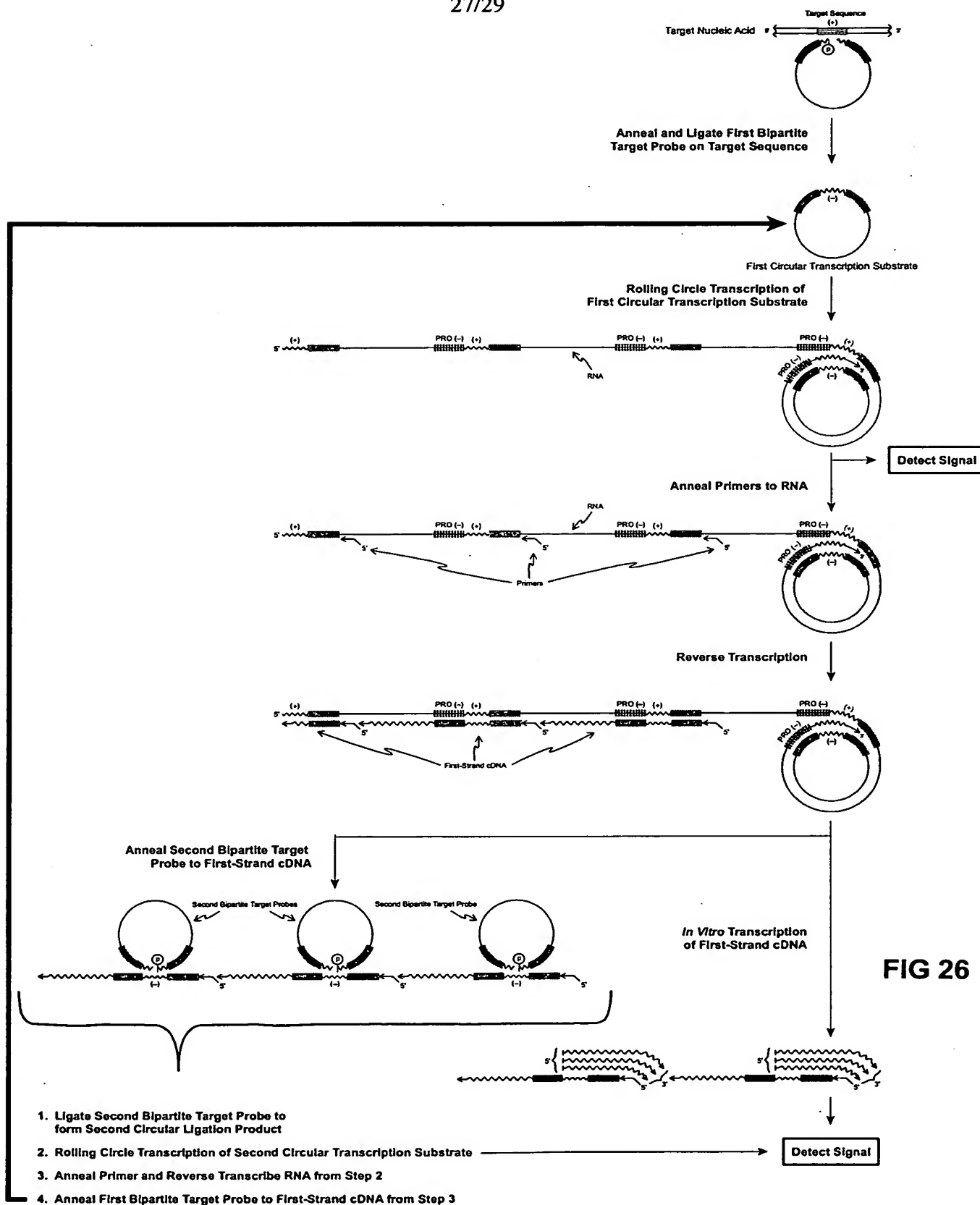


FIG 25

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 Application No.:  
 Docket Number: 310307.00006

27/29



28/29

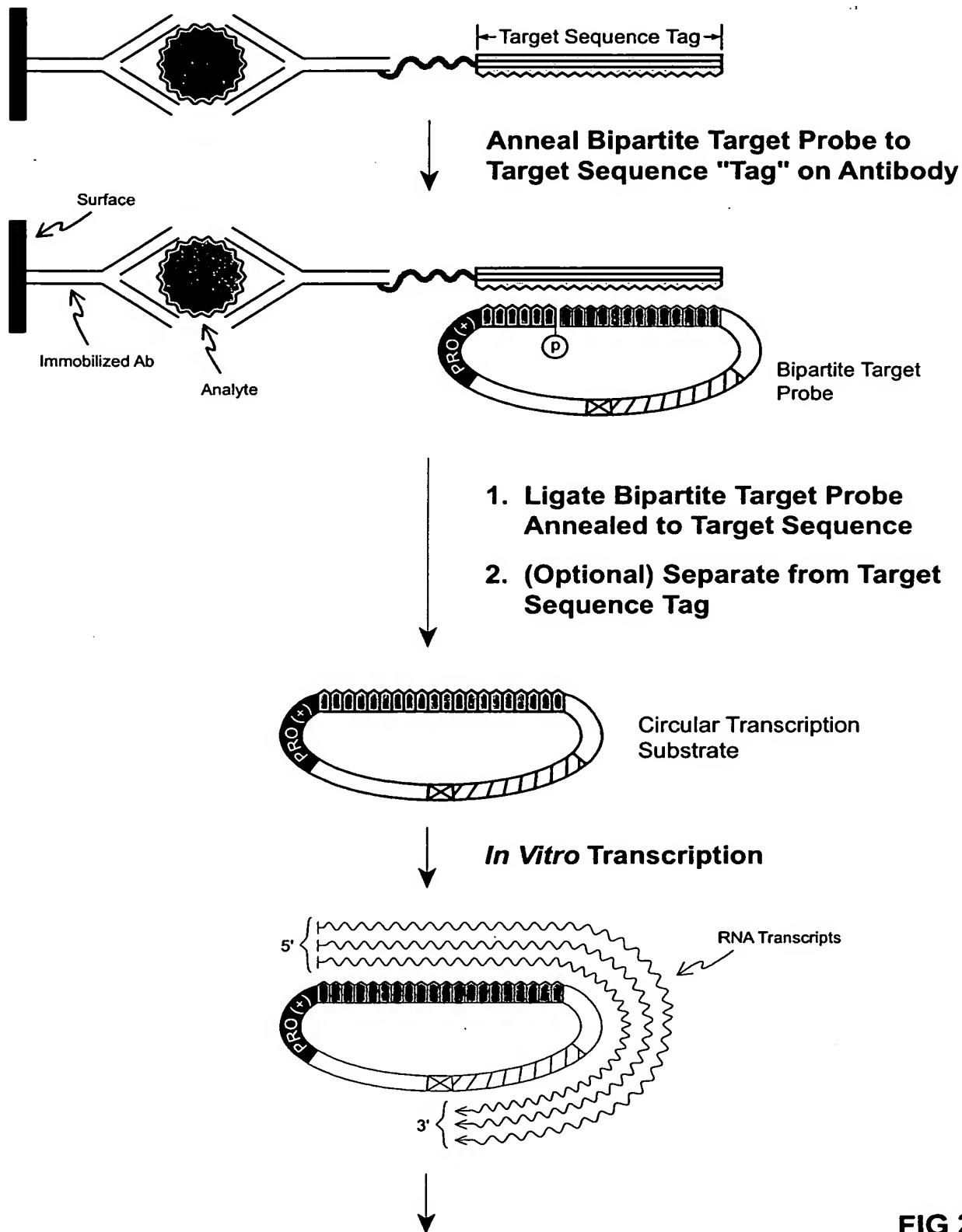


FIG 27

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29/29

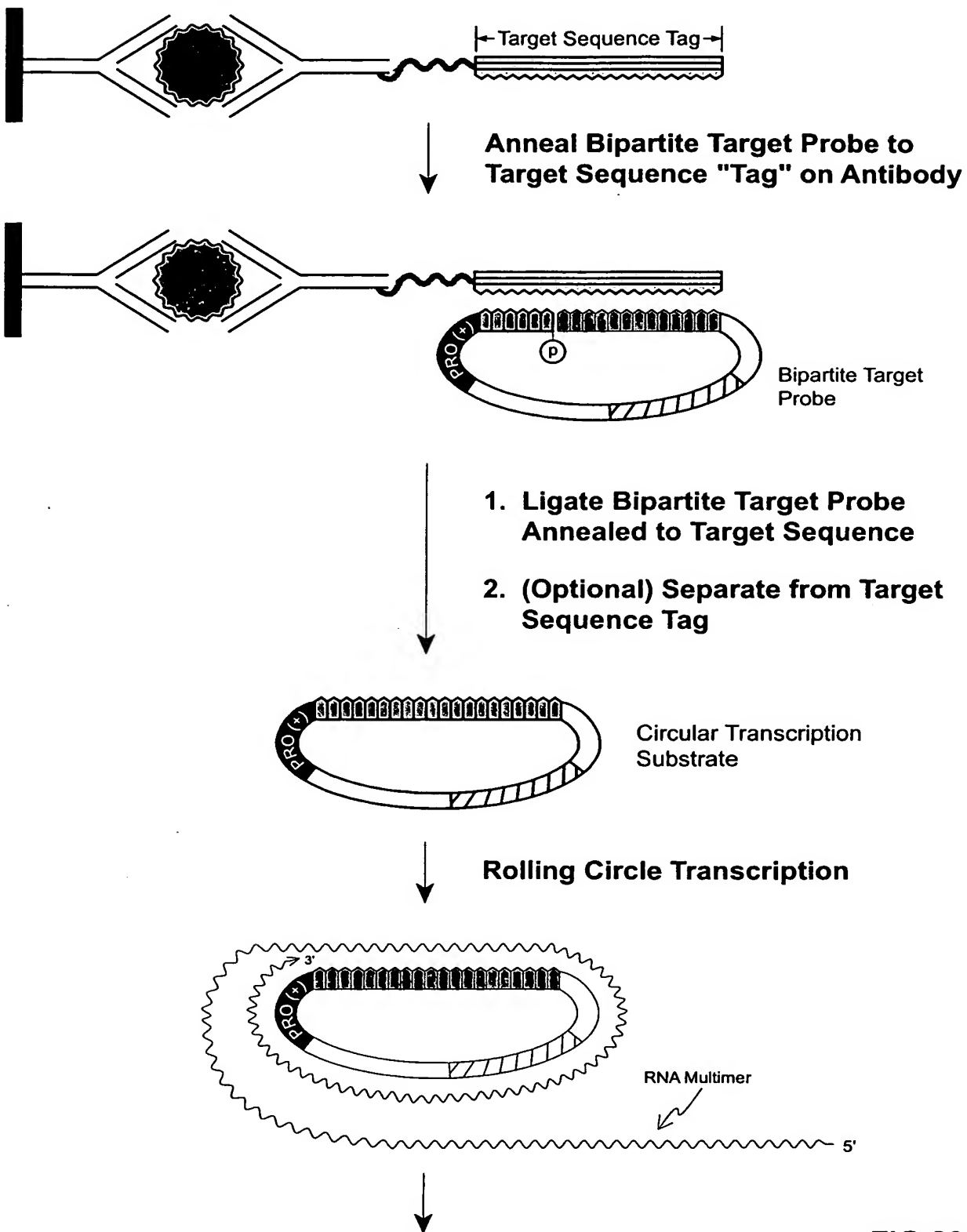


FIG 28

Detect RNA Transcription Products Directly or Indirectly